Monitoring and Mitigation to Address Fecal Pathogen Pollution along California Coast

Proposition 50 Coastal Management Program
California State Water Board Agreement No. 06-076-553

Applied Marine Sciences, Inc.

University of California Davis

California Department of Fish and Game
Marine Wildlife Veterinary Care and Research Center

Under the auspices of

Central Coast Long-term Environmental Assessment Program

CLEAN

Final Report

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1. Executive Summary

1.1. Report Organization

Coastal waters worldwide have been significantly influenced by human activities, as they are adjacent to densely populated areas and support a wide range of transport, commercial and recreational uses. Urbanization-associated impairments of nearshore water quality can result from enrichment of nearshore marine waters by nutrients and chemical and biological pollutants that are transported from terrestrial watersheds to the ocean in ever-increasing quantities. . Even after reaching the ocean this pollutant load poses health risks to humans and animals, and the degree of risk from marine dispersal of anthropogenic chemicals and pathogens may be greatly under-estimated. Fecal indicator bacteria (FIB) that normally reside in the gastrointestinal tract of humans and animals are used throughout the world to assess the microbiological quality of drinking and recreational waters. In the United States, FIB are used to define bacterial water quality standards aimed at reducing health risks in waters used for recreation and aquaculture. Groups of standard FIB monitored in water include total and fecal coliforms, *Escherichia coli*, and enterococci, and are considered as indicators of health risk in epidemiologic and quantitative microbial risk assessment (QMRA) studies.

To date, many monitoring programs have focused only on FIB measurements and do not test for the presence or absence of known pathogens, partly due to associated costs and expertise required for pathogen testing. However, substantial evidence has been collected challenging the usefulness of FIB data as a predictor of actual disease risk from contact with polluted water. A few limitations of using standard FIB to represent pathogens in water include the fact that FIB have the ability to multiply in the environment, they are not host-specific, and absence of FIB does not necessarily mean that pathogens are also absent. Consequently, alternative indicators of fecal pollution are needed that address the weaknesses of standard FIB for protection of human health. Ideally, these indicators would decay at similar rates as pathogens, be present at high concentrations in fecal sources, and be present at low concentrations in unpolluted environments. An added benefit of using alternative indicators is that in some cases host sources of fecal contamination can be identified.

The goals of this research program were to use both laboratory and field approaches to investigate issues related to water quality monitoring and mitigation of fecal pathogen pollution along the central California coast. Our specific objectives were to 1) evaluate water quality monitoring approaches by characterizing the relationships between FIB and enteric pathogen detection in a broad range of freshwater and marine surfacewaters along the central coast, 2) consider the relative importance of fecal pathogen loading from different sources, 3) evaluate whether filter-feeding estuarine or marine invertebrates (mussels) may be better indicators of water quality than direct water testing, 4) evaluate microbial source tracking techniques to distinguish between human and animal sources of fecal pollution, 5) characterize patterns of fecal pathogen shedding among terrestrial and marine animals, and 6) evaluate wetlands as a possible Best Management Practice (BMP) to mitigate impairments and improve surface water quality with respect to fecal pathogen pollution. The results are organized according to a series of priority questions that relate to the study goals and objectives, and are summarized in the following section, with additional details provided throughout the report.

1.2. Results for each Question

- *What are the spatial and temporal patterns in fecal indicator bacteria and pathogens along the central California coast, and what is the relationship between fecal indicator bacterial concentrations and fecal pathogen detection in: wastewater influent and effluent; rivers and streams; ocean and mussels; stormwater; and wetlands?*

Fecal indicator bacteria (total coliform, fecal coliform, *Enterococcus,* and *Bacteroidales* counts) were compared with direct detection of target bacteria (*Campylobacter* spp., *Salmonella* spp., *Escherichia coli* O157, and *Vibrio* spp.) and protozoa (*Giardia* and *Cryptosporidium*) in wastewater and surface water samples during a two-year period. Wastewater influent and effluent samples were tested quarterly from four wastewater treatment facilities. These facilities varied in the volume of wastewater handled per day, as well as the technologies utilized for wastewater processing. A significant reduction of pathogens between pre- and post-treatment was noted for all 4 facilities, but with differences among facilities in removal efficiency of FIB and enteric pathogens.

Water samples from ten coastal river sites were tested monthly over two years. FIB and enteric pathogen detection was both common and widely distributed between the ten coastal rivers, although detection was not highly correlated with sampling during the wet or dry seasons. Stormwater was sampled from three sites, and ocean water and mussels were sampled quarterly from six sites. FIB and specific pathogen detection was less common in these nearshore marine samples, when compared to river or stormwater. Pathogen trends in stormwater were similar to other sample types, with the protozoa *Cryptosporidium* and *Giardia* detected most often, followed by *Salmonella* and *Vibrio* spp., and little or no *Campylobacter* or *E. coli*-O157:H7 detection.

Quarterly testing of water collected from multiple sites in the Tembladero Slough constructed wetland showed that pathogens were detected most often in the slough sourcewater and at the inflow site, and less frequently as water moved down through the wetland. The ability to predict pathogen occurrence in relation to FIB threshold levels was evaluated using a weighted PQ measure that showed the universal *Bacteroidales* genetic marker had a comparable or greater mean predictive potential than standard FIB. We found that measures of traditional indicator bacteria, including coliforms and enterococci, correlated detection of some, but not all bacterial and protozoal pathogens in this study. Collectively our study findings suggest that monitoring for indicator bacteria alone may not provide sufficient information to minimize public contact with fecal pathogens in surface waters. We recommend utilizing a combination of FIB and specific pathogen assays to provide the most useful and accurate perspective regarding the presence, relative abundance, and contributing sources of fecal contamination in environmental water samples.

- *How can fecal pathogen loading of nearshore ecosystems be compared across the full range of surface water inputs to the ocean?*

This question emerged during the course of the study as a key synthesis question that could help resource managers more accurately assess local risks from water contact, and prioritize management strategies to minimize coastal pollution. We used data collected from different sources (i.e., streams and rivers, storm runoff and wastewater), combined with data on flow rates and the number of potential loading sources by area, to make preliminary estimates of relative loading and to identify gaps where future study and data compilation is needed to improve the risk estimates. In some cases, fecal pathogen inputs were identified from all three sources (streams and rivers, storm runoff and wastewater) and were not dominated by any single source. Given the inherent uncertainty in our load estimates and considering only the days on which sampling occurred, the average daily ocean input of *Vibrio cholerae* was significantly greater for streams and rivers, while wastewater was the major contributor for *Giardia.* In contrast, *Cryptosporidium* and *V. parahaemolyticus* inputs were not significantly different among sources. Collectively our data suggest that discharge of pathogens in water originating from streams, rivers and storm runoff poses a greater risk to human health than offshore, deep-water discharges of wastewater effluent along the central California coast due to the absence of water treatment, limited pathogen dilution, and direct shoreline discharge patterns associated with the inland surface water sources.

- *What is the relationship between exceedences of water quality objectives for fecal indicator bacteria (FIB) and fecal pathogen detection in surface water samples?*

California has set cutoffs for FIB counts to ensure public safety during water contact recreation and consumption of shellfish harvested from surface water bodies. In the current study, stormwater samples most commonly exceeded water quality (FIB) criteria, followed by river/stream/slough samples, and finally ocean water. Associations between FIB exceedances and specific pathogen detection varied by water sample type and pathogen group. Of all target pathogens, only *Cryptosporidium* detection was significantly associated with total coliform levels that exceeded current water quality criteria cutoffs. High fecal coliform counts were more closely associated with the presence of specific pathogens in surface water: *Cryptosporidium, Giardia, Salmonella*, and *V. parahaemolyticus* detection were all significantly associated with fecal coliform exceedences, while high enterococcal counts were predictive of *Giardia* and *V. parahaemolyticus* detection in surface water. These findings generally support the continued use of water quality criteria using FIB cutoffs for predicting health risks during recreational water contact and shellfish harvest. However, the lack of association between presence of some pathogens and FIB exceedences supports the concept that “absence of evidence is not necessarily evidence of absence”, meaning that enteric pathogens may still be present in surface waters with acceptable FIB levels, as was observed in the current study. This finding underscores the need to consider using multiple, or alternative water quality monitoring practices to improve our ability to predict pathogen presence and minimize health risks associated with water contact. Quantitative Microbial Risk Assessment is one framework that can be used to more comprehensively consider, characterize, and predict health risks associated with different beneficial uses.

- *Are mussels better indicators of ocean microbial water quality than seawater?*

In the current study we compared time- and location-matched mussel sampling with collection and processing of 20 L volumes of seawater. Shellfish, including mussels, clams and oysters have all been suggested as more sensitive bioindicators of water quality in aquatic ecosystems, when compared to water “grab” samples. However, using whole mussel homogenates we found no significant difference in pathogen detection between time- and location-matched mussels and seawater. For example, the prevalence of *Giardia* and *Vibrio* species detection differed by less than 2%. However, some notable differences were observed:

Campylobacter and *Salmonella* were detected in seawater when mussels tested negative, with 10% and 5% pathogen prevalence, respectively, in seawater. Similarly, *Cryptosporidium* oocysts were detected in 26% of seawater samples, but only 6% of mussel batches. Based on the weather and water quality characteristics present during our sampling efforts, we suggest that bivalves may be most useful as bioindicators when sampled during "high-risk" periods for fecal contamination of aquatic ecosystems, such as during or after storm events.

- *Which of three microbial source tracking methods is most promising and what can be learned about trends in human versus animal sources of fecal pollution?*

Evaluating microbial source tracking (MST) techniques for distinguishing human from animal sources of fecal pollution along the central California coast was deemed important because the approaches for remediating human as compared to animal sources of fecal contamination are different, and because new molecular approaches are providing insights on source tracking that were previously unavailable with traditional phenotypic characterization methods. The three MST methods evaluated were 1) *Bacteroidales* assays, 2) an *Enterococcus* surface protein (esp) gene assay, and 3) total to fecal coliform ratios in water samples. The comparative study showed that the *Enterococcus esp* assay and total: fecal coliform ratios did not perform as well and do not show as much promise as *Bacteroidales* for future MST work. Based on the comparative MST results, *Bacteroidales* host-specific qPCR was then used to quantify fecal bacteria in water and provide insights into contributing host fecal sources. More than140 surface water samples from 10 major rivers and estuaries within the Monterey Bay region were tested during 14 months with four *Bacteroidales*-specific assays (universal, human, dog, and cow). Bayesian conditional probability analysis was used to characterize the performance of *Bacteroidales* assays, and the ratios of concentrations determined using host-specific to universal assays indicated that fecal contamination from human sources was more common than livestock or dog sources in the coastal study sites.

- *What are the patterns and risk factors for fecal pathogen shedding from central coast animals, and are the same types of fecal pathogens detected in sea otters as are detected in other marine and terrestrial animals?*

Feces from domestic and wild animals were tested to determine the prevalence and genotypes of selected pathogens in the Monterey Bay region. Of 808 fecal samples tested between 2007 and 2010, 28% were positive for one or more target pathogens, and many of the same species detected in terrestrial animals were also isolated from sea otters. *Giardia* spp. were isolated most frequently, with an overall animal prevalence of 15%, followed by *Campylobacter* spp. (11%), *Vibrio cholerae* (9%), *Cryptosporidium* spp. (6%), *Salmonella* spp. (6%), and *Vibrio parahaemolyticus* (5%). Molecular characterization of *Giardia* and *Cryptosporidium* revealed both zoonotic and host-specific genotypes. Fifteen different *Salmonella* serotypes were detected, 11 of which were isolated from opossums, a non-native species introduced to coastal California. Risk factors associated with pathogen detection in animal feces included animal group, age class, gender, live-dead status, and season. These study findings provide insights that may be used to help prioritize animal management and water quality monitoring strategies.

- *Are wetlands effective in reducing fecal pathogen loads in surface water, and, if so, what wetland characteristics are most important to achieve pathogen reduction?*

Wetlands evaluation involved both controlled laboratory trials and field experiments. First, laboratory mesocosm tank models that simulated coastal wetlands were used to study specific variables believed to reduce the load of fecal pathogens present in contaminated runoff as it flows through a wetland. By introducing known quantities of specific pathogens at the inflow, and collecting samples under varying climatic and wetland restoration conditions (e.g., wetland length, vegetation configuration, salinity, flow rate), we determined the effects of these variables on reduction of pathogen concentrations in water traveling through the model wetlands. These studies revealed that the presence of vegetation enhanced removal of oocysts from fecally-polluted water at both fast and slow flow rates. The important role of vegetation in removal of waterborne protozoa should be considered in wetland reconstruction and management efforts for coastal ecosystems. Similar water measurements were conducted during quarterly testing at a reconstructed wetland at Tembladero Slough, providing a larger scale, "real world" model of the ability of coastal wetlands to reduce fecal pathogen loads in surface waters. These larger-scale findings indicate that both the distance from various point source(s) of contamination and periodic rainfall events influence the efficiency of pathogen reduction in natural systems.

Considered collectively, our study findings provide important new insights for water quality managers working at all levels and in multiple disciplines. These include specific suggestions for improving water quality monitoring and mitigation efforts in order to optimize the balance between coastal development and safety of coastal marine waters for recreation, shellfish harvest and other beneficial uses such as threatened and endangered species protection. Publications and outreach materials related to this project will be posted on the website www.pathogenpollution.org as they become available.